NATIONAL AERONAUTICAL AND SPACE ADMINISTRATION
GRANT NGR-14-005-050

# THE PHYSICAL AND CHEMICAL PROPERTIES OF HUMAN SWEAT AND FACTORS AFFECTING THE WATER BALANCE IN CONFINED SPACES

SEMI-ANNUAL STATUS REPORT NO. 1
FOR THE PERIOD: 1 July 1965-31 December 1965

from the
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## I. Personnel

For the period 1 July 1965-31 December 1965 Table 1 lists the personnel who worked under this grant.

## II. Research Aims

This project envisages a comprehensive study of the chemical and physical properties of human sweat produced by a combination of heat exposure and exercise on a motor-driven treadmill. We plan to fill in some gaps left by previous workers, with special emphasis on the correlation between chemical composition and physical properties. Ultimately we plan to study the physico-chemical properties of sweat from single glands, because only in this way can one avoid the statistical approach imposed by specimens of sweat collected from the whole body, in bags, or under small plastic cups. These represent populations of hundreds or thousands of glands, and may mask the physiology of the single gland.

During the first six months (1 July 1965-31 December 1965) we spent much time establishing protocols and validating methods.

## III. Collection and Processing of Sweat

At present the subjects are not kept under dietary, activity, or environmental control prior to an experiment; the questions under study do not require such elaborate control as yet. Both men and women are studied.

One day before an experiment, the subject cuts the finger nails and shaves both arms with an electric razor (Norelco, Universal, 2 rotors). On the morning of the experiment the subject scrubs both arms for 3 minutes using a surgical brush and a detergent soap ("Vel", Colgate-Palmolive Co., New York). This preparation is neutral in reaction and non-allergenic. After scrubbing, the subject rinses the arm one minute under flowing warm tap water, and then in a cylinder of demineralized water. The arm is then dried with surgical sponges that have been boiled 30 minutes in demineralized water and oven dried.

It is a curious fact that in the large scientific literature on sweat, virtually no studies have been made on how best to prepare the skin. Yet this is fundamental to any study. We propose to pay careful attention to this problem.

Vinyl plastic obstetrical gloves are donned to the elbow and held in place by elastic bands 2 cm in width. The subject then walks on the level at 6 km per hour for 100 minutes. Every 15 minutes a stop of 5 minutes is made to drain off sweat, and record the pulse rate and oral or rectal temperature. The room is kept at 40°C and 30 per cent relative humidity.

After walking the subject reclines for 15 minutes in a cool room (25°C, 50 per cent relative humidity) and if no symptoms persist, is discharged.

The volume of sweat is measured in calibrated cylinders. The samples are centrifuged at 2000 RPM for 10 minutes and the supernatant fluid decanted into vials of 10 ml capacity. These are cooled, the caps turned to a snug fit, sealed with masking tape, and refrigerated. If not used on the day of the experiment, they are frozen at -20°C until needed.

In each experiment the subject wears at the belt a "blank glove" containing 50 ml of distilled water. This blank is treated exactly like sweat and samples are carried through all procedures, so that appropriate blank corrections can be made if needed.

## IV. Chemical Composition of Sweat Compared with Osmotic Pressure (Freezing Point)

We are re-studying a problem reported on by Adams, Johnson, and Sargent (1958). When the osmotic pressure, calculated from the freezing point, was compared with the sum of important constituents, i.e. ammonia, sodium, potassium, chloride, lactate and urea, they found in some 600 specimens of sweat from young men that in about 1/3 of the samples the osmotic pressure derived from the freezing point was higher than that calculated by adding together the constituents. The findings of Adams, et al. (1958) could not be confirmed by the Oxford group (Foster, 1961), who were able to account for the osmotic pressure in their specimens by adding up the measured constituents.

In looking for an explanation of this discrepancy, it may be noted that the experimental conditions of the two laboratories were quite different. Our subjects were on a constant experimental diet. They walked out-of-doors in the summer and were completely acclimatized to moist heat. We had some 100 subjects. The Oxford subjects were not on a controlled diet, were caused to sweat by hot foot baths or by bicycling in a hot room, and were only 12 in number. It was possible that the Oxford group just did not happen to include anyone who produced the "unknown osmol" reported by the Urbana laboratory.

We have extended the experimental design in several ways. Men and women are included in the study. The specimens are analyzed within three days of collection. The nitrogen estimation includes total nitrogen, urea, and ammonia. Calcium, magnesium and various sulfur fractions are going to be studied. Acid-base balance is included.

Table 2 lists the methods which are being used in this current study.

The results to date are of considerable interest. We have collected specimens of both types--no "osmotic deficit" or a substantial "osmotic deficit". Examples of each are shown in Table 3. Generally speaking, all specimens whether from a man or a woman, are close to ionic balance. That is, the sum of $NH_4^+$, $Na^+$, and $K^+$ is approximately equal to the sum of $Cl^-$, $HCO_3^-$, and lactate$^-$, and sodium is usually approximately equal to chloride. There is always a residual nitrogen, i.e. the difference between total nitrogen and the sum of urea plus ammonia nitrogen, but in some specimens this residual nitrogen is large, in others small. So far it appears that in those specimens with a large osmotic deficit, there is also a large residual nitrogen.

We postulate now that the "unknown osmol" does exist in some specimens, and may be a nitrogenous, neutral compound that will have an osmotic activity, but not an ionic effect. If further studies confirm these preliminary results, we shall look into the residual nitrogen of the sweat, and attempt its fractionation.

Another preliminary conclusion is that the Oxford group just did not happen to have conditions or subjects that would permit detection of an "unknown osmol" in some specimens of human sweat.

## V. Acid-Base Balance and Human Sweat

Among the many aspects of sweat that have been given very little attention by previous workers is the acid-base balance. If bicarbonate or other buffers exist in substantial concentration, the sweat could play a role in acid-base balance and osmotic economy. We are studying two aspects of this problem--dissociation curves for $CO_2$, and titration curves from pH 2 to pH 13.

Our first approach is <u>in vitro</u>. In some experiments, sweat is used as it comes from the glove. In others, sweat is concentrated in a freeze dryer (Hansen and Robbins) to about 1/10 its original volume.

Appropriate samples are equilibrated in a gas proof collecting glove at 25°C, and the pH, $PCO_2$, and total $CO_2$ measured. By varying the $PCO_2$, a series of points can be plotted on a dissociation curve. Also, pK´ can be calculated. Table 4 shows such an experiment. At present, it looks as if sweat behaves like water, and is not a $BHCO_3/H_2CO_3$ buffer system.

There are very few reports on the titration curve of sweat, and yet this is a fundamental consideration when one thinks of the sweat as a physico-chemical system. Is it a buffer system? If so, what are the components?

Table 5 lists an experiment on one specimen of sweat concentrated by freeze-vacuum drying. Four inflection points can be found in most specimens. These are at about the following pH values: 2.6, 3.5, 10.0, and 11.5.

Three are perhaps identifiable; these may be ammonia with a pK´ of 4.76, lactate with a pK´ of 3.81, and carbonate with a pK´ of 10.36. The identity of the fourth is as yet a mystery.

## VI. Viscosity of Sweat

To the best of our knowledge, no measurement has ever been reported of the viscosity of sweat. By contrast, numerous studies of this physical property have been made on blood and synovial fluid. Yet the spread of sweat has a direct bearing on its wetting capacity, and thus on the evaporative heat loss, a fundamental component of the thermal balance equation.

We have chosen the capillary tube method as easiest and most appropriate for the present purpose. The instrument is that of Cannon & Fenske (1938), and is calibrated against water.

Table 6 lists the viscosity values for water, a series of sodium chloride standards, and a few specimens of sweat. One important fact has already emerged: the viscosity of sweat is variable from one specimen to another. This has the implication that the spread of sweat on the skin must also be variable. It also has the implication that the latent heat of vaporization of sweat is not just that of an equal weight of water, because viscosity and latent heat of vaporization have been shown to be functions one of the other (Ewell and Eyring, 1937). Thus a correction may be needed in the fundamental equations for heat balance when evaporative heat loss is to be calculated.

## VII. Studies on Individual Sweat Glands

We are just beginning to study individual sweat glands. To begin with, the cat's paw will be studied *in situ*. Later we hope to study individual glands in humans. We have just set up a dissecting microscope, a micromanipulator and a system for recording changes in pressure. No experimental data are ready for reporting, as we are still validating the method.

## VIII. Summary of Progress-- 1 July 1965-31 December 1965

1. A comparison has been made between the chemical composition of human sweat and its osmotic pressure (freezing point). In most specimens the sum of urea, ammonia, sodium, potassium, chloride, and lactate is close to the osmotic pressure calculated from the freezing point. However, in some specimens there is a difference, sometimes large, between the two. In other words an "osmotic deficit" exists because some "unknown osmol" has been measured. In those specimens with a large osmotic deficit, there is also a large undetermined residual nitrogen, i.e. total nitrogen minus urea plus ammonia nitrogen. Therefore, the "unknown osmol" may possibly be in the nitrogen fraction.

2. Titration curves for sweat have been studied. There may be four inflection points, three of which may be for ammonia, lactate, and carbonate. The fourth is unidentified as yet.

3. Dissociation curves for $CO_2$ have been made. Sweat behaves like water in this respect, and so the $BHCO_3/H_2CO_3$ system cannot be important in it.

4. The viscosity of sweat varies from specimen to specimen, and is higher than that of water at the same temperature. This has important implications for those portions of the thermal balance equation that have to do with wetting of the skin, and with the latent heat of vaporization of sweat. The latter is probably not the same as for an equal weight of water.

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TABLE 1. PERSONNEL ASSOCIATED WITH
NASA GRANT NGR-14-005-050.

| Name and Title* | Period of Association | Percentage of Salary Paid from this Grant |
|---|---|---|
| R. E. Johnson<br>Professor | 1 Sept. 1965-31 Dec. 1965 | 0 |
| F. Sargent, II<br>Professor | 1 July 1965-31 Dec. 1965 | 0 |
| Mrs. Frances Robbins<br>Biochemical Technologist | 1 July 1965-31 Dec. 1965 | 0 |
| T. Morimoto<br>Research Associate | 1 Sept. 1965-31 Dec. 1965 | 100 |
| Mrs. Keun Shil Shin<br>Research Assistant | 1 Sept. 1965-31 Dec. 1965 | 100 |
| B. Blase<br>Graduate Assistant | 1 Sept. 1965-31 Dec. 1965 | 0 |
| Mrs. Susan Kinney<br>Clerk-typist | 27 Sept. 1965-31 Dec. 1965 | 25 |

* In addition to those listed, there were research subjects who worked from time to time as needed. These included: E. Behling, F. Blase, J. Bodammer, K. Leoni, J. Miliszkiewicz, J. Nommensen, P. Molé, W. Berger, and U. Mazumdar.

TABLE 2. CHEMICAL METHODS FOR STUDYING THE COMPOSITION OF HUMAN SWEAT (NASA GRANT NGR-14-005-050).

| Substance | Authors | Type of Method |
|---|---|---|
| Total Nitrogen | Koch and McMeekin, 1924; Davenport, 1926 | Acid digestion, nesslerization, colorimetry |
| Urea Nitrogen | Van Slyke and Cullen, 1914 | Urease, aeration, nesslerization, colorimetry |
| Ammonia Nitrogen | Van Slyke and Cullen, 1914 | Aeration, nesslerization, colorimetry |
| Na, K | White, 1952; Baird Assoc., 1953 | Flame photometry |
| Chloride | Cotlove, Trantham, and Bowman, 1958; Am. Inst. Co., 1960 | Aminco-Cotlove titrator |
| Osmolarity | Wesson, 1952; Fiske Assoc., 1954 | Freezing point (Fiske osmometer) |
| Lactic Acid | Hawk, Oser, and Summerson, 1951 | Copper liming, colorimetry |
| Ca, Mg | Kovács and Tárnoky, 1959 | Chelation, titration |
| Viscosity | Fenske and Cannon, 1952 | Capillary tube |
| Total $CO_2$ | Consolazio, Johnson, and Pecora, 1963 | Manometry |
| $HCO_3$ | Consolazio, Johnson, and Pecora, 1963 | Manometry |
| S | Bertolacini and Barney, 1957 | Barium chloranilate, colorimetry |
| pH | Clark, 1928; Bates 1954 | Glass electrode |
| Freeze drying | Hansen and Robbins, 1956 | Vacuum plus solid $CO_2$ freezing |

TABLE 3. CHEMICAL COMPOSITION OF HUMAN SWEAT COMPARED WITH OSMOTIC PRESSURE (NASA GRANT NGR-14-005-050).

| Measurement or Calculation | | Sample of Sweat | | | |
|---|---|---|---|---|---|
| | | A (No osmotic deficit) | B | C (Large osmotic deficit) | D |
| $Na^+$ | mEq/L | 30.0 | 32.0 | 76.0 | 62.0 |
| $K^+$ | mEq/L | 5.7 | 5.3 | 5.8 | 8.4 |
| $NH_4^+$ | mEq/L | 3.7 | 3.3 | 2.9 | 4.7 |
| $Cl^-$ | mEq/L | 24.8 | 24.5 | 71.0 | 57.4 |
| $Lactate^-$ | mEq/L | 15.1 | 14.1 | 11.1 | 16.2 |
| $HCO_3^-$ | mEq/L | 0.0 | 0.0 | 0.3 | 0.0 |
| Urea | mM/L | 11.3 | 7.1 | 11.7 | 10.8 |
| Undetermined residual N | mAtoms/L | 5.9 | 12.4 | 16.6 | 8.9 |
| $\Sigma\ Na^+ + K^+ + NH_4^+$ | mEq/L | 39 4 | 40.6 | 84.7 | 75.1 |
| $\Sigma\ Cl^- + Lac^- + HCO_3^-$ | mEq/L | 39.9 | 38.6 | 82.4 | 73.6 |
| Ionic deficit | mEq/L | -0.5 | +2.0 | +2.3 | +1.5 |
| Osmolarity | mOsm/L | 89.5 | 86.7 | 190.8 | 177.3 |
| $\Sigma$ all solutes | mOsm/L | 90.6 | 86.3 | 178.8 | 159.5 |
| Osmotic deficit | mOsm/L | +1.1 | -0.4 | -12.0 | -17.8 |

TABLE 4. $CO_2$ DISSOCIATION CURVE OF ONE SPECIMEN OF HUMAN SWEAT AT 25°C. (NASA GRANT NGR-14-005-050).

| | $PCO_2$ mm Hg | pH | Total $CO_2$ mM/L | $HCO_3$ mEq/L | pK′ |
|---|---|---|---|---|---|
| A. | Pooled Sweat No. 2 | | | | |
| | 29.2 | 6.41 | 3.0 | 2.1 | 6.06 |
| | 55.5 | 6.22 | 4.1 | 2.3 | 6.11 |
| | 74.5 | 6.07 | 5.1 | 2.7 | 6.01 |
| | 103.4 | 6.05 | 6.5 | 3.2 | 6.06 |
| B. | Distilled Water | | | | |
| | 25.6 | 5.83 | 1.3 | 0.5 | 6.07 |
| | 58.0 | 5.95 | 3.3 | 1.4 | 6.08 |
| | 75.6 | 5.41 | 3.5 | 1.1 | 5.68 |
| | 106.0 | 5.58 | 5.2 | 1.7 | 5.89 |

TABLE 5. TITRATION CURVE OF HUMAN SWEAT*
(NASA GRANT NGR-14-005-050).

| Acid Range | | Basic Range | |
|---|---|---|---|
| N/10 HCl Added mEq $H^+$ | pH | N/10 NaOH added mEq $OH^-$ | pH |
| 0.000 | 7.56 | 0.000 | 7.52 |
| 0.025 | 5.49 | 0.025 | 8.78 |
| 0.050 | 4.61 | 0.050 | 9.29 |
| 0.075 | 4.24 | 0.075 | 9.66 |
| 0.100 | 3.92 | 0.100 | 10.06** |
| 0.125 | 3.71 | 0.125 | 10.59 |
| 0.150 | 3.50** | 0.150 | 11.03 |
| 0.175 | 3.25 | 0.175 | 11.24 |
| 0.200 | 3.02 | 0.200 | 11.38 |
| 0.225 | 2.83 | 0.225 | 11.45 |
| 0.250 | 2.64** | 0.250 | 11.55 |
| 0.275 | 2.48 | 0.275 | 11.60** |
| 0.300 | 2.32 | 0.300 | 11.63 |
| 0.325 | 2.25 | 0.325 | 11.70 |
| 0.350 | 2.14 | 0.350 | 11.75 |
| 0.375 | 2.11 | 0.375 | 11.76 |
| 0.400 | 2.00 | 0.400 | 11.76 |

Ten [redacted] ml [redacted] *The Sweat was pooled sweat No. 2. [redacted] were titrated with acid or base at 25°C.

**Point of inflection

TABLE 6. VISCOSITY OF WATER AT 24.3°C
SOLUTIONS OF SODIUM CHLORIDE, AND
SPECIMENS OF SWEAT.
(NASA GRANT NGR-14-005-050).

| Specimen | Density gm/ml | Viscosity Centipoises | Viscosity Relative to Water |
|---|---|---|---|
| A. Water | 0.99722 | 0.9080 | 1.0000 |
| B. Sodium Chloride | | | |
| 0.01M | 0.99790 | 0.9163 | 1.0091 |
| 0.12M | 1.00264 | 0.9176 | 1.0105 |
| 0.25M | 1.00791 | 0.9269 | 1.0208 |
| 0.50M | 1.01809 | 0.9517 | 1.0481 |
| C. Sweat Specimen | | | |
| JB | 0.99933 | 0.9290 | 1.0231 |
| EB | 1.00171 | 0.9264 | 1.0202 |